

Impact Assessment of Skin Irritation, Hematological Profile and Skin Sensitivity by SMA-DMSO

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ABSTRACT: SMA-DMSO (RISUG) is a high efficacy male anti-fertility drug, currently in clinical trial phase III. To evaluate the allergenicity of SMA-DMSO, *in vivo* skin allergenicity and sensitivity tests were conducted using Charles foster rats and guinea pigs. Skin Sensitization test was conducted in Charles foster rats in two phases – induction phase and Challenge phase. After the induction phase, there was a rest period of 2-weeks, during which an immune response could develop, and then the animals were exposed to a challenge dose. The extent and degree of skin reaction to the challenged dose in the test animals was compared with control animals which undergone sham treatment during induction and received the challenge exposure. Sensitization severity grading, based on incidence of positive responses, (i.e., irritation score of 1.00 or more) in comparison to control animals indicate that the test sample may be a Mild to Moderate sensitizer. The test sample of SMA–DMSO has been recommended as “Mild Irritant”, since the dermal irritation index was found to be 0.79 which as per the OECD indexing, is a mild irritant. Occlusive patching procedure has been used in test animals followed by investigation of erythema and odema in the period of 24 to 72 hours to assess skin sensitivity. Moreover, assessment of Haematological parameters in rats showed no variation in PCV, Leukocyte count and mean haemocrit value after injection of SMA-DMSO leading to conclusion that SMA-DMSO does not induce any kind of changes in haematological and histological profile of mammals.

KEYWORDS: SMA-DMSO, Skin sensitivity, Charles foster rats, Hematological profile, Irritation score

I. INTRODUCTION

SMA-DMSO complex, commercially available under the trade name of RISUG (Reversible inhibition of sperm under guidance) is currently in clinical trial phase III and it is recognized as potent male anti fertility drug which alters the pH of male vas deferens and ruptures the sperm, making it ineffective (Guha et al, 1997). Genotoxicity and Cytotoxicity tests conducted by different research groups under *in vitro* and *in vivo* experimental conditions on CHO cell line (Pallavi et al, 2013, 2014), Rhesus monkeys (Sethi et al, 1990) and albino rats (Sethi et al, 1992) have proved the safety and non mutagenicity of this complex on mammalian cell lines. However, skin allergenicity and dermal parameters changes induced by injection of SMA-DMSO have not been investigated by researchers. More focus has been put on analysis of clastogenic and genotoxic impacts induced by RISUG.

Commission's Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) issued a guidance document (SCCNFP, 2000) that has the purpose to provide guidance for testing cosmetic ingredients and for the safety assessment of the finished product, has made it mandate to assess skin sensitivity and irritation tests compulsory in alternate animal models before launching the product for human use. Although the SCCNFP pursues the aim of replacing *in vivo* sensitisation tests with *in vitro* alternatives, it concedes that no validated alternatives are available to date (SCCNFP, 2000). It therefore requires cosmetic ingredients to be tested in an animal test according to OECD and EU test guidelines and according to Good Laboratory Practice (GLP).

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Many skin sensitisation tests in guinea pigs (e.g., Draize test, open epicutaneous test, optimisation test, split adjuvant test) are available. Mouse Local lymph node assay (LLNA) has emerged as a promiscuous strategy to check skin sensitivity based on evaluation of the stimulation index. In the LLNA, the test substance is applied onto the mouse ear on three consecutive days. On the sixth day, the proliferation of lymphocytes in the draining lymph node, caused by the primary immune reaction, is measured. This is done usually by measurement of the incorporation of ³H-methyl thymidine into the DNA of proliferating lymphocytes in draining lymph nodes. The LLNA does not include a challenge phase. The endpoint of interest is the stimulation index giving the ratio of thymidine incorporation in lymph nodes from dosed animals compared to the incorporation in lymph nodes from vehicle-treated control animals. The test is positive when the stimulation index ≥ 3 (Kimber et al., 1986; 1989; Kimber et al., 2002).

The MEST (mouse ear swelling test) comprises both the induction phase and the elicitation phase of the immune response. (Asherson and Ptak, 1968; Gad, 1986; 1994). Several weeks prior to and during the test period mice are fed a diet enriched in vitamin A since this has been shown to enhance contact sensitization. Ear thickness of test and control ears is measured under ether anaesthesia with a micrometer 24 and 48 hours after application of test substance. Ear swelling is expressed as the difference between test and control ears in percent.

The MEST has been evaluated independently by several laboratories and in inter-laboratory studies. It was concluded that the MEST is a useful model for identifying strong contact sensitizers. (Cornacoff et al., 1988; Hignet et al., 1989; Dunn et al., 1990).

Current research work was conducted to assess the effect of SMA-DMSO injection on haematological parameters, Dermal index and skin sensitivity using Charles foster rats and guinea pigs.

II. RELATED WORK

Sethi et al, 1990 conducted the safety evaluation studies of RISUG in rats reporting no significant toxicity on haematological parameters and body weight after injecting RISUG for duration of 3 months at the dosage level of 3 mg to 5 mg. Later, Singh et al in 1992, evaluated the potential of RISUG in rhesus monkeys at the dose of 100 mg (contraceptive dose, CD), 250 mg (CD x 2.5) and 500 mg (CD x 5.0), and the monkeys were kept under observation for one year. The observed behavioural, haematological, biochemical and histopathological parameters in treated monkeys were comparable to controls. Their findings suggested that the polymer SMA-DMSO is safe up to 5 times Contraceptive dose in monkeys. In current time, RISUG has been considered 100% safe in clinical trial phase 1, 2 and 3 when injected in vas deferens of animals and human beings, still lot of questions have been raised over the possibility of induction of genetic level changes by RISUG in long term. Different groups of scientists have raised concerns over chromosomal damage possibility and probable risk of skin allergenicity consequences by injection of RISUG in vas deferens of males.

Cytotoxicity of SMA-DMSO was assessed using COMET assay whereby concentration of SMA-DMSO ranging from .001 μ M to 1 μ M was tested for inducing any significant DNA damage in Mammalian (Chinese Hamster Ovary) cell line (Pallavi et al, 2013). 50 cells were scored for each concentration, with 25 cells scored from each of two replicate blind coded slides. Statistical significance of the results was assessed by means of one-tailed Student's t-test. Analysis of tail length and % DNA amount after treating the CHO cells with varying concentration of SMA-DMSO, ranging from 0.001 μ M to 1 μ M has shown no significant DNA damage and cytotoxicity. Results have shown RISUG to be absolutely safe for further usage in Mammalian cells.

Multigeneration teratogenicity studies on RISUG- injection were carried out by Sethi et al in Charles Foster rats. Observations parameters – gonadal function, estrus cycles, mating behaviour, conception rates, early stages of gestation, organogenesis, gestation period, labor and delivery, litter size, litter weights during different days of weaning and lactation, were not effected. After analysis of the data generated in three segmental studies upto three generations, it was concluded that RISUG – injection did not cause any teratogenic effect in Charles Foster rats, at the doses used in the experiment (Singh and Sethi; 2002) Phase I, II and III clinical trials of RISUG- injection in humans are likely to be completed in different centres of India (Guha, 2002).

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III. METHODOLOGY OF SKIN IRRITATION TEST

The skin irritation potentials of sample “SMA.-DMSO” (provided by M/s Glenmark Laboratories Ltd.), was assessed in male rabbits. Animals were obtained from CDRI Animal facility and maintained under standard husbandry conditions. Standard Pellet diet and water were provided to the animals ad libitum. Skin Irritation Test was conducted according to OECD Guideline No 404.

Preparation of Patch Site: Approx. 24 hour before the test, fur of the dorsal area of trunk was removed by close clipping with electric clipper. Care was taken to avoid abasing of skin and only those animals were used which had healthy intact skin.

Procedure: Occlusive patching was used. 0.5 ml of test sample was applied to the test site of skin respectively. Test sample was applied to the cotton gauge, which was placed over the shaved clean skin and covered by aluminium foil. Patch was held in place with cotton bandage to avoid access by the animal to the patch site and to prevent ingestion or inhalation of the test sample. At the end of exposure period (4 hr.), patch was removed. Observations were made at 1, 24, 48 & 72 hours after removal of the patches. Erythema and oedema were evaluated and scored as per OECD Guideline.

SKIN SENSITIZATION TEST

SMA-DMSO was tested for skin sensitization potentials in male guinea pigs. The test was conducted as per OECD guideline no. 406. Animals were obtained from CDRI Animal breeding facility. Male guinea pigs were maintained under standard husbandry conditions and provided pellet diet, and water. The animals were initially exposed to the test substance by epidermal application. Following a rest period of 14 days, during which an immune response may develop, the animals were exposed to a challenge dose. The extent and degree of skin reaction to the challenge exposure in the test animals was compared with that demonstrated by control animals which undergo sham treatment during induction and receive the challenge exposure.

Preparation of animals and procedure

Animals were acclimatized to the laboratory conditions for one week prior to the test. Before the test, animals are randomized and assigned to the treatment groups. Approximately 24 hour before the test, fur was removed by close clipping with electric hair clipper. Care was taken to avoid abrading of skin. Animals with healthy intact skin were used. The sample (RISUG) was applied by occlusive patching to a small area of approximately 6 cm² on the shaved skin and covered with cotton gauge which was again covered with aluminium foil. The patch was held in place with cotton bandage to avoid access by the animal to the patch site and to prevent ingestion or inhalation of the test compound. At the end of exposure (5 hours), patch was removed and the animals were examined for signs of erythema and edema. 20 animals were used in the experimental group and ten animals in control.

Test conditions :

The test was divided into two parts -(a) Preliminary test and (b) Main test.

Preliminary Test: It was conducted to determine dose levels -(i) the dose that caused moderate to severe erythema and (ii) the dose that did not caused erythema.

Main Test: During induction phase, animals were treated with 0.08 gm of RISUG once a week for three week, to induce immune response of the animals. The animals were allowed for two weeks of rest and then challenged with 0.02 gm of RISUG. The extent and degree of skin reaction to the challenge exposure in the test animals is compared with

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that demonstrated by control animals which undergo sham treatment during induction and receive the challenge exposure.

Observations

Observations were made at 24, 48 and 72 hours after removal of the patches. Erythema and edema were evaluated and scored as per OEC D Guidelines.

IV. RESULTS AND DISCUSSION

RESULTS FOR SKIN IRRITATION TEST

In skin irritation test, Dermal irritation index in adult male rabbits at the dose of 0.5 ml was found to be 0.79. Dermal irritation index of 0.0 is a non – irritant, 0.0 to 0.5 is a negligible irritant, 0.5 to 2.0 is a mild irritant, 2.0 to 5.0 is a moderate irritant and 5.0 to 8.0 is considered to be a severe irritant. In the present studies, the dermal irritation index was found to be 0.79.

	*R-1				*R-2				*R-3			
	Cont		Expe		Co		Expe		Con		Expe	
	ry	ed.	ry	ed.	ry	ed.	ry.	ed.	ry.	ed.	ry.	ed.
1 hr.			3	2			3	2			3	1
24 hr.			1				2					
48 hr.							1					
72 hr.							1					
Irritation	0		0.75		0		1.125		0		0.5	
Range	0.5 to 1.12											
Dermal Irritation Index	0.79											

*Rabbit Number Ery = Erythema Oed = Oedema

Table 1: Dermal irritation index of Charles foster Rats after 1 hr- 72 hr post treatment with SMA-DMSO

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Analysis of skin erythema and oedema between the control group and experimental group of Charles Foster rats between 1 hr to 72 hr post treatment with SMA-DMSO shows that there is no significant change in the dermal irritation index and only a mild irritation level can be seen in the experimental group as reflected by the observed intensity.

RESULTS ON HEMATOLOGICAL PARAMETERS AFTER TREATMENT WITH SMA-DMSO IN CHARLES FOSTER RATS

On measurement of body weight, no changes in weight of these rats were observed after 15 days of injection of SMA-DMSO in vas deferens. No significant variation in total Red blood cell count and MCV was observed in group II, III and IV of albino rats as compared to control rats after injection of SMA-DMSO.

S.NO	Parameters	Groups	Initial	Final
1.	Haematocrit (%)	I (control)	43.36±2.26	43.2±3.52
		II	45.1±2.641	44.72±1.23
		III	44.38±2.714	43.55±2.13
		IV	45.82±2.18	46.82±2.61
2.	MCV	I (control)	55.56±1.43	56.63±2.29
		II	60.9±3.10	59.81±2.34
		III	59.68±2.45	60.12±1.66
		IV	59.92±3.82	59.81±2.45

Table 2 : Effect of SMA DMSO on Hematological parameters in Albino rats (Mean± S.D)

Hematocrit value and Mean Corpuscular Volume (MCV) observed in control and treated groups I to IV of Charles albino rats indicate the count of erythrocytes and leukocytes remains almost same even after injection of SMA-DMSO in vas deferens of these rats in the concentration range .01 M to 1 M. In treated group II, injected with .01 μM SMA-DMSO, initial Hematocrit value observed was 45.1 which was coherent with control group having Hematocrit value 43.36.

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RESULTS FOR SKIN SENSITIVITY TEST

Following tables show the impact on skin of guinea pigs after administration of SMA-DMSO After 24 hrs, 48 hrs and 72 hours. Average irritation score was calculated at the end of the process.

Animal No	Irritation at 24 hours	Irritation at 48 hours	Irritation at 72 hours	Irritation Score
1	1	+1	+1	1.00
2	0	+1	+1	0.66
3	0	+1	+2	1.00
4	0	+1	+1	0.66
5	+1	+1	+1	1.00
6	0	0	+1	0.33
7	0	+1	+1	0.66
8	+1	0	0	0.33
9	0	+1	+1	0.66
10	+2	+1	+1	1.33
11	+1	+1	+1	1.00
12	+1	+1	+1	1.00
13	+1	+2	+1	1.33
14	0	+2	+2	1.33
15	0	+2	+1	1.00
16	0	+3	+1	1.33
17	+1	+2	+1	1.33
18	0	+1	+1	0.66
19	0	+1	+1	0.66
20	0	0	0	0.00

Table 3: Effect of RISUG (SMA-DMSO) on skin sensitization in Male guinea pigs in experimental group and control group

Dermal irritation index of 0.79 indicates that RISUG can be considered as a mild skin irritant as per the Draize system and scoring. Average irritation score in the 20 samples of guinea pigs is in the range of .06-1.00 which is in synchronous with the skin sensitivity results obtained on Charles foster rats showing that SMA-DMSO is absolutely safe in terms of skin allergenicity and sensitivity.

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V.CONCLUSION

Hematocrit value and Mean Corpuscular Volume (MCV) observed in control and treated groups I to IV of Charles albino rats indicate the count of erythrocytes and leukocytes remains almost same even after injection of SMA-DMSO in vas deferens of these rats in the concentration range .01 M to 1 M. In treated group II, injected with .01 μ M SMA-DMSO, initial Hematocrit value observed was 45.1 which was coherent with control group having Hematocrit value 43.36. After 3 months of injection of rats in group II, III and IV with .01 μ M, 0.1 μ M and 1 μ M concentration of SMA-DMSO respectively, the Hematocrit value observed initially was 45.1, 44.38 and 45.82 while after 3 months, it was 44.72, 43.55 and 46.82 respectively. As there is no significant variation in haematological parameters, body weight and clinical behaviour of all the 3 treated experimental groups of Charles foster strain of albino rats as compared to control group after injection of RISUG, it can be inferred that it is absolutely safe for further clinical trials. Average irritation score obtained in guinea pigs after 24, 48 and 72 hrs of skin exposure to SMA-DMSO was in the range 1.3 which signify no dangerous impact or allergic effect on Skin. Moreover, skin allergenicity tests prove that SMA-DMSO has no allergenicity induction potential, it can be processed further for assessment of other toxicological safe parameters. Skin irritation, sensitivity and haematological profile assessment tests prove that SMA-DMSO is a mild skin irritant and does not cause any changes in erythrocytes and leukocytes count in animals when injected *in vivo*. It can be processed further for assessment of other toxicological safe parameters.

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